CLAIMS

1.– Pulsed field electrophoresis chambers of TAFE (Transversal alternating Field Electrophoresis) or CHEF (Contour Clamped Homogeneous Electric Field) systems for separating DNA molecules by means of using a system for energizing their electrodes and alternating the direction of application of the electric field as well as a system for circulating the buffer, which chambers comprise:

i) single or several useful electrophoresis zones (UEZ), each supporting a minigel,

ii) dimensions that depend on the separation 'd' between the pairs of electrodes with opposite polarity, as well as on the number and sizes of the UEZ, being said separation between the electrodes from 6.2 to about 15 cm, and being the chambers able to analyze a number of samples that also depends on 'd' and on the number and sizes of the UEZ,

iii) the absence of non-useful electrophoresis zones (NEZ), being the NEZ excluded from the chamber using materials of high dielectric constant,

iv) stretched electrodes, that are pulled tight by the action of a fixation system in CHEF chambers and by a fixation and tension system in TAFE chambers,

v) TAFE chambers that could be in inverted TAFE configuration.

2.- Electrophoresis chambers as claimed in claim 1 wherein the CHEF chambers have single UEZ that can support a rectangular or square shaped minigel

3.- Electrophoresis chambers as claimed in claim 2 wherein the dimensions of the rectangular-shaped minigel of CHEF chambers are d/3 cm in length and d/1.732 cm in width, being the width from 3.6 to about 8.7 cm, and the length from 2.1 to about 5 cm

4.- Electrophoresis chambers as claimed in claim 2 wherein the length and the width 'a' of the square-shaped mini-gel of CHEF chambers is d/3 cm, being 'a' from 2.1 to about 5 cm.

5.- Electrophoresis chambers as claimed in claim 1 wherein the NEZ were excluded from the CHEF chambers, so the area of the floor of the chambers is calculated from the knowledge of 'd', according to the formula $[2 +(d/0.87)]\bullet[6 + d]$, being said area from about 111.3 to about 404.1 cm^2.

6.- Electrophoresis chambers as claimed in claim 1 wherein the TAFE chambers are of width 'L', being said width the dimension parallel to the cathodes and anodes and up to 50 cm, and being possible to subdivide the TAFE chambers in two or a larger number of UEZ, each supporting a minigel, division that is done along said 'L' dimension.

7.- Electrophoresis chambers as claimed in claim 1 wherein the length of the minigels of TAFE chambers is d • 0.515 cm, length that is from 3.2 to about 7.7 cm.

8.- Electrophoresis chambers as claimed in claim 1 wherein said chambers use minigels of width 'a', minigels that can analyze in a single electrophoresis run a maximum number of samples which is calculated as (a – 0.2) / 0.25.

9.- Electrophoresis chambers as claimed in claim 1 wherein the NEZ are eliminated from TAFE chambers, so the side walls, supporting the gel and the electrodes, have an area that is calculated from the knowledge of 'd' according to the formula [2 + 1.4 • d]•[2 + 0 54 • d] – 1.02 •[1 + 0.54 • d]^2, being said area from 37.8 to about 149.5 cm^2.

10.- Electrophoresis chambers as claimed in claim 1 wherein the UEZ of TAFE chambers are the result of subdividing the chamber evenly along the electrodes, so the minigels are placed sequentially one next to the other.

11.- Electrophoresis chambers as claimed in claim 1 wherein the TAFE chambers can be of type I or type II.

12.- Electrophoresis chambers as claimed in claims 1 and 11 wherein the type I TAFE chambers have all UEZ arranged in a fixed or removable single platform that has continuous electrodes of length 'L'.

13.- Electrophoresis chambers as claimed in claims 1, 11 and 12 wherein the type I TAFE chambers have all minigels supported in a single frame or each minigel independently placed in an UEZ, for which said chambers must have laterally grooved pieces to slide said minigels.

14.- Electrophoresis chambers as claimed in claims 1 and 11 wherein the type II TAFE chambers have the UEZ placed in fixed or removable independent mini-platforms, each having a minigel and electrodes, being the arrays of electrodes physically separated among the UEZ, but able to be plugged in parallel to acquire continuity; so, when the chamber is energized with a single power supply, all samples loaded in all minigels are at the same electrophoresis conditions.

15.- Electrophoresis chambers as claimed in claim 1 wherein the TAFE chambers have activated only the minimum number of UEZ that are required to analyze the number of samples desired, and have excluded from the electrophoresis the non-used UEZ, exclusion performed by inactivation or occlusion of UEZ with properly shaped pieces made of any material with high dielectric constant; being the number of active UEZ from 1 to the maximum number of UEZ.

16.- Electrophoresis chambers as claimed in claim 1 wherein TAFE chambers can be subdivided in a number of UEZ that ranges from 1 to 30.

17.- Electrophoresis chambers as claimed in claim 1 wherein said TAFE chambers can be in inverted TAFE configuration, configuration that has the cathodes of the miniplatforms at the bottom of the electrophoresis chamber and the anodes at the top, thus being the samples loaded in the minigel bottom, so, the samples migrate in the direction opposite to the gravity.

18.- Electrophoresis chambers as claimed in claim 1 wherein the TAFE chambers have the NEZ excluded from the electrophoresis run, being the exclusion achieved either by occluding parts of the chamber with materials with high dielectric constant, or by constructing the external chamber walls parallel to the imaginary plane containing the cathode of one electric field and the anode of the other electric field, being these walls the ones that do not support the electrodes and being they placed at most 2 cm apart from said imaginary plane.

19.- Electrophoresis chambers as claimed in claim 1 wherein the electrodes, which are kept fixed by the action of a fixation system in CHEF and TAFE chambers, enter into the chamber from the outside, are energized with a single power supply during the electrophoresis and enter in contact with the buffer passing through the bores of elastic plugs inserted into holes drilled in the floor of CHEF chambers or in the walls of TAFE chambers supporting the gel, said plugs being used to fix the electrodes to the chamber

20.- Electrophoresis chambers as claimed in claims 1 and 19 wherein the elastic plugs through which the electrodes pass can be made of silicone, rubber or any other elastic material.

21.- Electrophoresis chambers as claimed in claim 1 wherein TAFE chambers have a system to pull tight the electrodes crossing the walls of said chamber, system which is placed at the exit of each electrode, being said system comprised of.

i) a rod that is slotted in its top side, rod that is able to turn and has a waist-shaped notch crossed by a hole into which the end of the electrode is inserted and bent around the rod waist,

ii) a grub screw which sets definitely the rod in the desired position

22.- Accessories for pulsed field electrophoresis chambers comprising:

i) a removable system for attenuating the turbulences of the flowing buffer solution,

ii) a disassemblable system for casting electrophoresis gels of flat surfaces, with the absence of irregularities or meniscuses between the wells where the sample plugs will be loaded into, wells that are formed in the gel with the aid of comb-shaped pieces, each having several identical teeth, and existing various sets of combs that differ in the width and thickness of their teeth,

iii) a disassemblable set for forming sample plugs of homogeneous dimensions, dimensions similar to those of the gel wells where the plugs will be loaded into, having said system different blocks.

23.- Accessories for pulsed field electrophoresis chambers as claimed in claim 22 wherein the removable system, for attenuating turbulences of the buffer solution flowing throughout

CHEF chambers, has two types of rectangular sheets: the A and B type sheets, both made of any material with high dielectric constant, sheets that are as wide as the inner part of the chamber, being the A type at least 2 cm in height, and the B type 0.5 cm in height

24.- Accessories for pulsed field electrophoresis chambers as claimed in claims 22 and 23 wherein the A type sheets are placed over the chamber floor and separated from it from 0.02 to about 0.05 cm, sheets that protrude from the buffer in the chamber, so the buffer circulating throughout the chamber can only flows through the gap formed between the inferior edge of the A type sheets and the floor of the chamber.

25.- Accessories for pulsed field electrophoresis chambers as claimed in claims 22 and 23 wherein the B type sheets are glued to the floor of the chamber and fully submerged in the buffer, so the buffer circulating throughout the chamber only flows over the B type sheets

26.- Accessories for pulsed field electrophoresis chambers as claimed in claims 22 and 23 wherein both types of sheets are alternately arranged from the buffer inlet and outlet toward the electrode array of the chamber in the following order: an A type sheet followed by a B type sheet, repeating 'n' times said pair of sheets, being 'n' an integer between 1 and 4, and placing the last sheet about 1 cm apart from the electrodes and being said last sheet of A type.

27.- Accessories for pulsed field electrophoresis chambers as claimed in claim 22 wherein the accessories for attenuating turbulences of buffer solution flowing throughout TAFE chambers are two identical sheets made of a material with high dielectric constant, sheets of the same size as the walls of the chamber, sheets placed in parallel to the plane containing the electrodes of the same gel side, said sheets having a horizontal slot in the inferior third, being the length of the slot equal to the width of the chamber and the height up to 0.5 cm.

28.- Accessories for pulsed field electrophoresis chambers as claimed in claims 22 and 27 wherein the sheets for attenuating turbulences of buffer flowing throughout TAFE chambers are placed, one near to the buffer inlet, and the other near to the outlet, thus

dividing the chamber in three compartments: the central one, containing the UEZ, and two lateral ones through which the buffer is delivered into the chamber or is withdrawn from it

29.- Accessories for pulsed field electrophoresis chambers as claimed in claim 22 wherein the disassemblable system for casting gels of flat surfaces is comprised of:

i) a flat base plate,

ii) two frames from 0.35 to about 0.5 cm in thickness, one of them with a rectangular-shaped cavity and the other with a square-shaped cavity, having both two notches for inserting a comb with long teeth, being the frame thickness and the internal cavity dimensions, the ones that determine, respectively, the thickness of the gel and the width 'a' as well as the length of the gel that will be used as supporting medium in the electrophoresis in CHEF or TAFE chambers,

iii) a comb with long teeth, teeth that determine the dimensions of the wells where the sample plugs are loaded into,

iv) two covers, a cover 1 which fits against the front of the comb, and a cover 2 which fits against the back of the comb,

v) a second comb, similar to the comb with long teeth but having shorter teeth for pushing and aligning the sample plugs loaded into the gel wells.

30.- Accessories for pulsed field electrophoresis chambers as claimed in claims 22 and 29 wherein the comb with long teeth is flat in the frontal part whereas in the rear and over the teeth it gains thickness forming a step, being all teeth identical and from 0.03 to about 0.1 cm in thickness, from 0.15 cm to the gel width minus 0.3 cm in width, and equal to the gel thickness minus 0.1 cm in length.

31.- Accessories for pulsed field electrophoresis chambers as claimed in claims 22 and 29 wherein the comb with short teeth has shape and dimensions similar to the ones of the comb with long teeth, excepting the length of teeth, which is about 0.2 cm shorter.

32.- Accessories for pulsed field electrophoresis chambers as claimed in claim 29 wherein the cover 2, or cover fitting against the rear of the comb, has two flat surfaces and a protruding edge, whereas the cover 1, fitting against the front of the comb, has two flat surfaces but one of its edges has a bevel cut in wedge formation.

33.- Accessories for pulsed field electrophoresis chambers as claimed in claim 22 wherein the disassemblable set for forming sample plugs of dimensions that are homogeneous and similar to those of the wells of the gel where they will be loaded into, said set that is comprised of:

i) several sample plug makers, each composed by a flat impermeable block, thicker than 0.5 cm, block that has several parallel grooves lengthwise, being the width of each groove 0.2 cm, and the depth equal to the teeth thickness of a given comb, being said depth from 0.03 to about 0.1 cm, and existing plug makers for all possible teeth thickness of the combs with long teeth that can be used to form the gel wells,

ii) a flat rigid and impermeable sheet of at least 0.1 cm in thickness, which acts as the cover of the sample plug block,

iii) several sample plugs cutters, each being a bar that is as long as or longer than the grooves of the block of the sample plugs maker, said cutters having legs in the ends which confer them an inverted-U shape, said cutters having several protuberances with cutting edges in its inferior part, said protuberances protruding 0 1 cm from the bar, said cutting edges being transversal to the longest dimension of the bar and 0.2 cm in length, said cutting edges being evenly spaced a distance that is from about 0.15 to the gel width minus 0.3 cm.

34.- Methods of use of the pulsed field electrophoresis chambers and the accessory systems for the separation of DNA molecules, and being given that the chamber is connected by a tubing system to circulate the buffer solution through an external heat exchanger, and that the electrodes of the chamber are energized by a system that also alternates the electric field direction, said methods of use comprise:

I. a method of use of the disassemblable set for forming sample plugs of dimensions that are homogeneous and similar to those of the wells of the gel where they will be loaded into

II. a method of use of the disassemblable system for casting gels of flat surfaces,

III. a method of use of the chambers and accessories for performing the electrophoresis process,

IV. a method for pulling tight the electrodes of TAFE chambers.

35.- Method as claimed in claim 34 wherein the disassemblable set for forming sample plugs of dimensions that are homogeneous and similar to those of the wells of the gel where they will be loaded into, comprises the following steps:

i) preparing a cell suspension in molten agarose gel and keeping it at 45 °C,

ii) pre-warming the grooved block, of the sample plug maker, and its cover at 45 °C,

iii) pouring said suspension in the grooves of the block,

iv) covering the grooved block with its cover-plate and maintaining the set at room temperature or at lower temperature until the agarose solidifies,

v) aligning the sample plugs cutter lengthwise on the first groove of the block with the protruding cutting edges turned downward,

vi) pressing down the sample plug cutter and further removing it from the set.

vii) tilting the grooved block and pushing the sample plugs into a vessel containing the proper solution for their treatment,

viii) repeating the process for all agarose strips solidified in all grooves of the block.

36.- Method as claimed in claim 34 wherein the disassemblable system for casting gels of flat surfaces comprises the following steps:

i) placing the frame on the flat base plate,

ii) fitting the legs of the comb with long teeth into the notches of the frame, or notches milled in the outer sides of the frame,

iii) placing the cover 1 on the frame and in front of the comb, with the flat surface turned to face the frame, the bevel edge against the comb,

iv) clamping the set until the interstices are sealed,

v) maintaining the molten gel between 65 and 70 °C,

vi) pouring the molten gel into the cavity, filling the cavity formed between the frame, the flat base plate and the cover 1,

vii) placing the cover 2 on the frame, introducing the protruding edge of the cover into the rear step of the comb with long teeth, thus eliminating the excess of molten agarose,

viii) leaving the system to set until the gel is solidified,

ix) removing the comb with long teeth, leaving the wells of the desired width and thickness formed in the gel,

x) placing the sample plugs on the wedge-shaped edge of the cover 1 and pushing said plugs with an applicator to slide them into the wells,

xi) placing the comb with short teeth in the set, by fitting into the notches of the frame the legs of said comb, then pushing said sample plugs to the bottom of the wells,

xii) removing the cover 1, the cover 2 and the frame from the set.

37.- Method as claimed in claim 34 wherein the process of electrophoresis, that will analyze 'x' number of samples in chambers that have multiple UEZ, comprises the following steps:

i) defining the minimum number of UEZ where 'x' samples can be loaded into,

ii) occluding the UEZ that will not be used in the electrophoresis,

iii) plugging in parallel the electrodes of the UEZ that will be activated. If type II TAFE chamber will be used.

38.- Method as claimed in claim 34 wherein the process of electrophoresis, that will be performed in minigels with flat surfaces of the desired dimensions, comprises the following steps:

i) connecting the chamber to the electric field alternating device and to the electrode energizing device,

ii) filling the chamber with 0.5X TBE buffer solution (1X TBE: 89 mM Tris, 89 mM Boric acid, 2 mM EDTA, pH 8.3),

iii) connecting the chamber to the external heat exchanger,

iv) checking the proper arrangement of the system for attenuating turbulences of the buffer flowing throughout the chamber,

v) circulating the buffer until the desired temperature is reached in the solution filling the chamber,

vi) interrupting buffer circulation,

vii) immersing the minigel or minigels into the chamber,

viii) restoring buffer circulation,

ix) calculating the electrophoresis time needed to separate the DNA molecules,

x) energizing the system, and performing the electrophoresis maintaining the buffer circulating throughout the chamber at high flow velocity.

39.- Method as claimed in claims 34 and 38 wherein the CHEF chambers require that the buffer level surpasses at least 0.3 cm the gel thickness, being it accomplished by adding volumes of buffer that can be calculated from the knowledge of 'd', or separation between the electrodes with opposite polarity, according to the formula {[2 + (d / cos (30°))] • [6 + d]} • (0.3 + gel thickness), being the added volumes from about 72.3 to about 323 3 ml of buffer solution if the gels are from 0.35 to 0.5 cm thickness.

40.- Method as claimed in claims 34 and 38 wherein the TAFE chambers require that the buffer level surpasses at least 0.3 cm the gel height, being it accomplished by adding volumes of buffer that can be calculated from the knowledge of 'd', or separation between the electrodes with opposite polarity, and the number of active UEZ ($NZUE_{active}$) in the chamber according to the formula

$[(2 + 1.4 \bullet d) \bullet (2 + 0.54 \bullet d) - 1.02 \bullet (1 + 0.54 \bullet d)^2] \bullet L \bullet NZUE_{active}/NZUE_{total}$, being said volumes from about 63.2 to about 7390 ml.

41.- Method as claimed in claims 34 and 38 wherein TAFE chambers that have a single active UEZ and CHEF chambers admit electric field strengths up to 25 or 16 V/cm, respectively, provided the chambers are energized using power supplies with a maximum power output of 300 watt and the buffer solution is maintained at constant temperature, being it from 4 to about 30 °C.

42.- Method as claimed in claims 34, 37 and 38 wherein TAFE chambers that have several active UEZ admit electric field strength from 8 to 25 V/cm, electric field that depends on the number of UEZ activated, provided the buffer is maintained at constant temperature, being it from 4 to 30 °C

43.- Method as claimed in claims 34 and 38 wherein the electrophoresis time in CHEF chambers is calculated from the knowledge of 'm' and according to the formula [(D/m)•2•tp], formula demanding to be fed with said distance 'm' that a selected lineal DNA molecule migrate during each pulse of duration 'tp', being calculated said running time values by specifying the distance 'D' in cm that the smallest molecule is wished to migrate in the gel, taking as the preferred 'D' value the one that equals the separation between the migration origin and the inferior gel edge minus 0.1 or 0.2 cm, provided the gel size was

calculated from the separation 'd' between electrodes with opposite polarity and being the electrophoresis run time, for separating DNA molecules up to 2 megabase pairs in 1 5% agarose, 0.5X TBE, 30 °C, from about 1.5 to about 9 hours at 16 and 5 8 V/cm, respectively, whereas at 10 °C it is from about 2.5 to about 14.5 hours at 16 and 5 8 V/cm, respectively.

44.- Method as claimed in claim 34 wherein the method of use of the system for pulling tight the electrodes of TAFE chambers comprises the following steps:

i) loosening the grub screw that fixes the rod into which the electrode is inserted,

ii) turning the rod the required angle for pulling tight said electrodes,

iii) tightening the grub screw to set the rod in the position that maintains the electrode stretched.